Note on Shoguna rufotestacea, *Lew.* (Ann. Mag. Nat. Hist. ser. 6, vol. iv. 1889, p. 274).

Fairmaire in 1886 described a species of *Shoguna* under the generic names of *Holocephala* and *Pachycephala*, both of which were preoccupied, and in the Pascoe collection there are several species standing under the MS. name of *Thione*. The genus seems to be represented by very numerous species in many parts of the tropical and subtropical zones. In the figure referred to above there are five joints given to the hind tarsus, but under a high microscopic power only four are visible.

LIV.—*On the Nutritive and Excretory Processes in Porifera.*

By Arthur T. Masterman, B.A., late Scholar of Christ's College, Cambridge, Assistant Professor and Lecturer on Zoology at the University of St. Andrews.

An abundance of young colonies of many of the commoner sponges in St. Andrews Bay gave opportunity for an investigation into the processes of nutrition and excretion as carried on in the group Porifera.

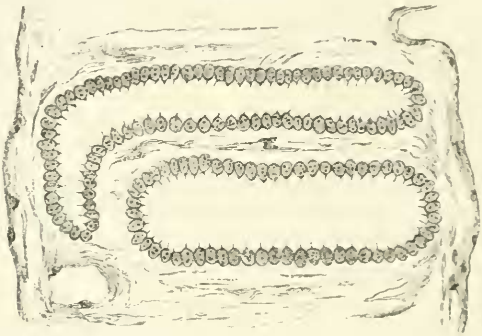
The experiments described below were carried out with small colonies of *Grantia compressa*. Particles of carmine were found to give the best result, although other colouring-matters were tried.

The colonies were all placed in sea-water with fine carmine particles in suspension, and allowed to remain there for two minutes; they were then removed, washed, and placed in sea-water.

At the expiration of short periods of time, varying from fifteen seconds to forty-five minutes, single colonies were removed, killed in osmic acid, and sectionized. It was hoped that a series of this kind would present a gradation of the changes undergone by the particles during digestion and excretion; but, partly owing to the difficulty of washing effectually, and so keeping extraneous carmine from being absorbed, and partly owing to a different rate of absorption and digestion in colonies varying in size, the sections do not show so distinct a gradation as was expected. The general course of the metabolic circuit through the sponge can, however, be clearly made out.

In figure 1, as in all the earlier sections, the carmine particles are seen to be confined to the choanocytes, and in the first series fifteen seconds after being removed from the carmine solution the choanocytes are all well charged with particles. A minute examination brings to light just a few particles here and there in the endodermal pinnacocytes, which are, at any rate, sufficient to show that these cells are at least capable of absorbing substances, though there are too few to allow us to assign a digestive function to them.

Fig. 1.



Transverse section of *Grantia*, showing loaded choanocytes.

There can be no doubt that in *Grantia* at least the choanocytes form the active ingestive tissues.

In all the series up to about three or four minutes the choanocytes still have carmine in their substance, but later than this they gradually lose it, until as late as ten or twelve minutes they are practically free of all particles. Amongst the charged choanocytes we notice that here and there are cells which have commenced to lose the definite cell-outline which distinguishes the normal choanocyte, and, later, to lose their flagella. Stages in this process of phagocytic degeneration can be observed, and although at first cells undergoing this change are scattered here and there in the endodermal chambers, yet in some of the series there are whole chambers which have been reduced by this process to the state of an amœboid multicellular mass, in which the only indication of the former cellular condition is the row of cell-nuclei dotted about in its substance.

Such an appearance is shown in figure 2 *b*, where the amœboid mass is seen to be in direct protoplasmic connexion with the mesodermal elements; and there is every reason to interpret this appearance as a wandering inwards of the transformed choanocytes. In fig. 2 *a* is seen an endodermal chamber in which some of the cells have not reached so advanced a stage of "transformation" as others, and some of these former still retain their cell-outline.

By selecting suitable examples we can compile a complete series showing the gradual transformation of a choanocyte to the amœboid condition. Such a series is represented in fig. 3.

Fig. 2.

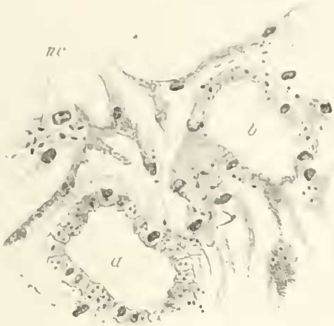


Fig. 3.

Fig. 2.—Transverse section of *Grantia*, showing two radial chambers and nephrocytes.

Fig. 3.—Series A, B, C, D, showing transformation of a choanocyte; camera, high power.

References.

a. Radial chamber, with progressing transformation.
b. Radial chamber, with amœbiform choanocytes.
nc. Nephrocytes.
v. Vacuoles around food.

Fig. 3 *a* is a choanocyte (drawn by camera under high power) showing the contained carmine particles. This is the flagellated ingestive phase. In *b* we find that the cell-outline has become less distinct, and this is especially so at one end, which is the inner end or the end away from the flagellum. This condition becomes more pronounced in the phase drawn at *c*, and, finally, at *d* we have the cell in the amœboid digestive phase, practically unrecognizable from an amœboid mesodermal cell.

Concurrently with this change the cell leaves the flagellated chamber-wall and migrates into the mesoderm. What ultimately becomes of it in this part of the sponge is very difficult to make out.

The transformed choanocyte either completes the course of intracellular digestion itself, or it is devoured by mesodermal phagocytes together with its contained nutritive particles. The choanocyte, however, at this stage so closely resembles the mesodermal cells that its ultimate fate cannot be followed in sections.

We have certain cases described which lead one to suppose that the cells may be devoured by phagocytes. Thus Dendy (4) figures a phagocytic cell in close contact with a choanocyte, and I have observed a few appearances in these sections which appear to be phenomena of a like nature. Delage (3) found that in the larva of *Spongilla* the "ciliated" cells are engulfed in the indifferent amœboid cells, and some at least are digested; the others, on the other hand, emerge later and form the endodermal pinnacocytes and choanocytes. These and other instances show that it is not an unknown phenomenon for the choanocyte to be eaten by the phagocytes.

At the same time there is not sufficient evidence in this sponge for such an assumption, and perhaps the real process is that intracellular digestion is conducted by the incurrent choanocyte, but that if this laden cell is unable, through an excess of nutrient material or through a low metabolic activity, to conduct the processes of digestion itself, it falls a prey to the mesodermal cells, just as do degenerating tissues in the phenomenon of phagocytosis occurring in the development and life-history of many forms.

The radial chambers appear always to be lined almost uninterruptedly with choanocytes in all later stages of digestion, and we may therefore conclude from this that the vacated places of the immigrating choanocytes are filled up either by re-transformed choanocytes or by mesodermal cells which assume the collared phase. One or both of these processes must obviously take place in order that the sponge may not be reduced to an amœboid mass without radial chambers. This result has actually been obtained in several cases cited below, when the sponges had been overfed.

We thus find that:—

1. The process of ingestion is conducted *almost entirely* by the choanocytes.
2. These choanocytes travel to the interior of the colony, and in doing so assume the shape of mesodermal phagocytes.
3. The process of digestion is conducted in the so-called

mesoderm either intracellularly by the loaded choanocytes or in some cases perhaps by a phagocytic digestion of choanocyte and its contents.

4. The immigrated choanocytes are replaced in the wall of the chambers by cells from the inner tissues, which assume the shape of choanocytes.

The continual morphological transformation above indicated appears to be the normal process of digestion in, at any rate, the simpler sponges.

To this statement two objections present themselves:—

Firstly, Sollas (15) has already, when criticising experiments conducted under similar conditions, taken exception to them, remarking that "there is at present no proof that carmine is a food, or that if it is sponges will readily feed upon it." This objection of course holds good with the above in common with all other feeding experiments, and they will be of no value if carmine be *proved not* to be a food.

Assuming it to be not so, it seems difficult to understand why the choanocytes should so very readily absorb large quantities of it. Minute particles of sand or other mineral matter if taken at all by the choanocytes must be very rapidly ejected, as their presence in these cells cannot be demonstrated; at least I have, after repeated attempts, been unable to do so. This being so, it is not too great an assumption to suppose that the choanocytes can and do exert a selective power, and are not obliged to absorb and ingest anything and everything which is presented to them, provided it fulfil only the necessary physical requirements of size &c. Again, it can be easily shown that numbers of small animals (young starfishes &c., Ruffer (12)) can and do feed upon carmine when it is presented to them, or, at any rate, they behave to carmine in exactly the same way as they do to any nutrient material.

Lastly, there is not wanting evidence that the carmine particles undergo a certain amount of change in the tissue of the sponge. Lendenfeld (9) finds that the larger particles, after having been expelled from the tissues, have lost their angular outline, and adduces this observation to show that they have at least undergone some change in their passage through the tissue of the sponge. I have also observed that in many of the loaded amœboid cells there are vacuoles developed around the enclosed particles (fig. 6), perhaps indicating that a process of digestion is going on.

Taking these facts into consideration, it does not seem to be too great an assumption to suppose that most animal organisms react towards carmine particles in the same way as they do towards undoubted foods. It is evident that this is all we require to assume for our purpose, without discussing the point as to whether a sponge, for example, can be kept alive for an indefinite period by feeding it upon carmine exclusively.

The second objection which might be urged is that the choanocytes were overfed, and that therefore the phenomena above described are of a pathological nature.

In this connexion Hæckel (6) has observed that in many calcareous sponges he found specimens in which the flagella had atrophied and the flagellated cells had assumed the amœboid state.

Metschnikoff (10) finds and describes allied phenomena in *Ascetta clathrus*, and he also notes that feeding with an excess of carmine causes obliteration of the chambers in *Halisarca pontica*, the whole interior of the colony being reduced to an amœboid mass.

Carter (2), Lieberkühn (8), and others give instances of the same kind.

Sedgwick (13), after quoting some of these instances, remarks:—"The collared cells are thus inconstant, and appear to be merely parenchyma-cells specially modified under certain conditions and capable of passing back into their original form when the need for them has passed away."

Sollas (14) mentions an appearance like that of a collared cell budding off an amœboid cell into the mesoderm.

Bidder (1) states that in *Ascetta* the collared cells wander through the ectoderm and, becoming perforated, form a pore; and there are numerous other cases in the development of sponges which show that the collared cells arise from amœboid and are readily transferred into either stage. The apical growth of sponge-colonies probably proceeds on the same lines.

All the cases above cited, in which the transformation of collared cells is effected in the adult sponge, are usually regarded as pathological, and so they probably are in the sense that they are the result of normal processes driven to an extreme; and it does not follow that all transformation of the collared cells upon feeding must necessarily be pathological.

In these experiments care was taken to avoid as far as possible all unnatural factors. Freshly obtained apparently healthy colonies were used, and attempts were made to avoid an excess of carmine either as to quantity or size of the particles. There is also no appearance in the sections which would point to overfeeding, all the collared cells being uniformly charged with a fair amount of minute carmine grains.

The fate of the loaded amœboid cells can be easily followed. In sections of colonies which were killed as late as or later than five minutes from the time of removal from the carmine (or seven minutes from the first immersion in the latter) the ectodermal outline is seen at places to be interrupted, as in fig. 2 or fig. 4, by a protrusion of the mesodermal substance to the exterior, with the appearance of a volcanic crater, and at some of these points are clearly seen a number of amœboid cells, which we may term "nephrocytes," charged with carmine particles and evidently in the act of leaving the sponge.

Fig. 5 shows a pair of these cells which were free in the paragastric cavity, seen under high power.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 4.—Mass of nephrocytes escaping from ectoderm.
Fig. 5.—Mass of nephrocytes free from colony.
Fig. 6.—Amœboid digestive cell.

These are mesodermal cells or metamorphosed choanocytes (it is obvious that it is quite impossible to pronounce definitely whether they are really the actual choanocytes which ingested the carmine in the incurrent canals), which are destined, upon the processes of digestion being completed, to leave the colony, carrying with them the solid waste products of excretion. We have here an example of a process of *intracellular* excretion for the removal of waste solids, quite distinct and apart from the liquid excretory processes which doubtless take place through the medium of the water-currents caused by the choanocytes.

A similar intracellular excretion is well known to exist in many Metazoa, as shown, for instance, by Durham in *Asterias* and by other observers in *Phyllirhoë*, *Bipeunaria*, &c.

The clear recognition of this intracellular excretion as occurring throughout the Metazoa concurrently with the liquid intercellular excretion would perhaps go far to explain the morphological differentiation of the excretory organs in this group, and seems to have a direct bearing upon the origin and significance of the cœlom.

The "nephrocytes" find their way to the exterior, so far as can be seen, *mainly* through the "ectoderm" or outer limiting layer of the colony; but there is no doubt that many also break through into the paragastric cavity, or even into the incurrent canals. A number of these loaded nephrocytes may be seen lying just under the outer layer, and they might under some circumstances, as referred to above, be mistaken for amœboid cells which are performing the function of *ingesting* carmine.

From the above facts we can now summarize the processes of digestion and excretion in *Grantia compressa* as follows:—

(1) Ingestion of food-particles into the substance of the choanocytes, or, at any rate, mainly into them, the possibility of their absorption by other parts, such as the ectoderm or endoderm, being undoubted, but practically of no importance compared with the former.

(2) Morphological transformation of the choanocytes into amœbiform cells, in no way differing in appearance from so-called mesoderm-cells.

(3) Immigration of these cells to interior, where intracellular digestion takes place. This stage is probably concurrent with stage (2).

(4) Replacing of the immigrated cells by fresh choanocytes arising from morphologically transformed mesoderm-cells.

(5) Excretion of the solid waste particles by amœboid nephrocytes, which burst through the limiting layer of pinnacocytes (mostly the outer layer, but not confined to that part) and leave the colony, probably to disintegrate.

In very briefly comparing these results with those of other observers we note that Carter (2) describes having traced the course of carmine-particles in the young *Spongilla*, and remarks that they are absorbed by the "spongozoa" (choanocytes), and that at the expiration of fifteen minutes the particles are again set free by these cells. Although he does not speak with any great certainty upon the course taken by these particles, he seems convinced that both in this form and in *Teichonella labyrinthica* the "spongozoa" are the actively ingesting layers.

Sollas (15) has also verified these observations upon colonies of *Spongilla*, stating that the choanocytes alone take up the particles of carmine.

Heider (7) experimented upon species of *Oscarella*, and he seems to have been led to the same result.

Von Lendenfeld (9) conducted a series of feeding experiments upon the Aplysinidæ, and his results led him to believe that the ingestion was conducted by the amœboid mesoderm-cells lining the subdermal cavities, and that the particles after digestion by these cells were ejected into the flagellated chambers and driven out by the choanocytes. He finds that the choanocytes do absorb carmine particles, but believes that these are soon ejected unchanged.

It is quite possible that concurrently with the development of subdermal cavities there is a change in the distribution of the digestive function; but it is difficult to believe that the choanocytes should perform the function of ingestion (apart from that of digestion) in so many simple sponges, and that they should not only lose this function in Aplysinidæ, but should actually take on the special one of assisting excretion.

It is worth noting that the metabolic circuit which I make out to be normal in *Grantia* is, allowing for the absence of subdermal cavities in this form, exactly the reverse of that in the Aplysinidæ, as observed by Von Lendenfeld.

This is worth mentioning, because in early experiments I found that I had not been careful to avoid the entrance of accidental particles of carmine after removal from the solution, and also had not subjected the sponges for a short enough time to the action of the carmine mixture, and hence the later stages (of excretion) might easily have been mistaken for early stages of ingestion. I think it therefore possible that, if the Aplysinidæ of the above-mentioned investigator were left in the carmine for a longer period than is required to complete the metabolic circuit, the sections following this experiment might equally well be interpreted as indicating that the carmine particles were absorbed by the choanocytes and ejected by the subdermal amœboid cells.

Lendenfeld certainly seems to write with great conviction, but some of his sponges appear to have been left for a long time in the carmine, *e. g.* as much as a quarter of an hour. It is remarkable in what an exceedingly short time the particles are scattered throughout the mesodermal parts of the colony, at least in *Grantia*.

On the other hand, it is possible that, together with the morphological differentiation of subdermal cavities, there may be a physiological change of function, and that, as suggested by Sollas (15), the phenomena described by Lendenfeld may be allied to those of inflammation in higher Metazoa. One may grant that the subdermal amœboid cells may have a function of ingestion of foreign bodies, without assigning to them the main function of the nutrition of the colony.

In a later work Lendenfeld (9) comes to the conclusion that carmine is only deposited in the amœboid cells quite exceptionally, as, for instance, in the case of lesion of the outer layer of cells, and that under normal circumstances it is the choanocytes only which absorb the carmine.

These statements and results are criticized by Metschnikoff (11), who points out that Lendenfeld grants that fat-globules are taken in by the mesodermal phagocytes; and he also declares that Lendenfeld's figures give "direct indications of the presence of carmine grains in the amœboid cells of the mesoderm."

He remarks also as follows:—"Although *it has not so far been definitely ascertained how the foreign particles penetrate the mesoderm after they have reached the interior of the sponge*, yet it has been clearly shown that they are largely absorbed by the mesodermic cells themselves. . . . Grains have been enclosed by the endodermic cells as well as by the amœboid phagocytes of the mesoderm.

"In certain sponges there are very few mesodermic cells, which consequently take a small part in englobing foreign bodies; in others, again, especially in the siliceous kinds, the mesoderm is much better developed, and its more numerous cells can therefore take in a proportionately larger number of these minute particles. There are a few species, such as the *Siphonochalina coriacea*, whose mesodermic cells alone enclose all foreign bodies, so that the cylindrical cells of the endoderm merely serve to keep up the continuous passage of the fluid through the sponge."

I have attempted to supply the deficiency indicated above by the italicized words of Metschnikoff, at least for *Grantia*, and I think it most probable that in the least differentiated sponges, e. g. *Ascetta*, we have so-called "endodermic" cells which perform at least two functions, ingestion and digestion, and that they change their form from flagellate to amœboid according as they perform the one function or the other. In the higher differentiated sponges the "mesodermic" cells become divided off to a greater degree into two morphological types concurrently with a more complete division of physiological labour—so much so that, as Metschnikoff indicates above, some forms occur in which there are choanocytic cells performing *only* the function of locomotion (of the particles).

These cells we should expect to find remaining constant throughout life, and they thus form a real *epithelium* of cells. The function of digestion, on the other hand, is here entirely performed by the mesodermic cells. Hence within the sponge group we find a process of physiological division of labour connected with the processes of ingestion and digestion, which may largely account for the morphological differentiation as seen in the various types. The smaller the proportion of the choanocytic endodermal area to the pinnacocytic area the less part (we shall expect to find) the individual cells of the former will take in the digestive processes and the more they will be differentiated into a definite "tissue" performing as a predominant function throughout life that of ingestion—that is to say, *intercellular* ingestion into the canals of the sponge and *intercellular* excretion through the osculum of the same, whereas their former function was that of *intracellular* ingestion, digestion, and excretion.

In the sponges, then, there is *intercellular* ingestion and excretion, as in all Metazoa, but there is no *intercellular* digestion, at any rate in the lower forms, a very important feature in comparing them with the Cœlenterata.

The Porifera would also appear to really consist of two layers only, one definite and fixed—the ectoderm—and the other, Metschnikoff's meso-endoderm, consisting of unspecialized cells, any of which may give rise to ova, spermatozoa, locomotive, excretory, skeletal, or digestive phases which have their corresponding temporary morphological modifications.

It is interesting to note that the probable process of digestion indicated above shows a very low type of Metazoan metabolic processes. Any one of many cells in close contiguity engulfs solid food particles, which it reduces to the liquid state, or the parts of them that are capable of being so reduced, by digestive processes. It then parts with most of its liquid nutrient material to the surrounding cells by diffusion or a modified process of a like nature, and is expelled or emigrates from the colony, carrying with it the solid waste products.

This probably represents the scheme of all *intracellular* digestion, and a modified form of it is found in the processes of ingestion as conducted by the "yellow cells" of Annelida, as also in the numerous phenomena of amœboid ingestion in higher Metazoa.

The discussion of the origin of the Porifera and their relationship to the Choanoflagellata on the one hand, and to the Cnidaria on the other, must be left to specialists in this group; but the facts shown above certainly seem to have a direct bearing upon this difficult question. The fact that the inner layer cells assume mastigopod and myxopod stages under different physiological conditions strengthens the resemblance between the simpler sponges and the colonial Choanoflagellata, and the absence of *intercellular* digestion (with the morphological differences necessarily correlated to this) points to a fundamental difference between the former and the Cnidaria.

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LV.—*Descriptions of Eight new Species of Butterflies from New Britain and Duke of York Islands, in the Collections of the Hon. Walter Rothschild and Mr. Grose Smith, captured by Captains Cayley Webster and Cotton.* By H. GROSE SMITH.

Papilio Websteri.

Male.—Upperside. Both wings black. Anterior wings with a curved row of six white spots between the veins towards the apex, the first above the costal nervure narrowly